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Effects of Longer Droughts on Holm Oak *Quercus ilex* L. Acorn Pests: Consequences for Infestation Rates, Seed Biomass and Embryo Survival

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Abstract: The effects of climate change on oaks *Quercus* spp. constitute a main environmental concern for the conservation of temperate forests. In this context, we assessed the consequences of longer droughts on the interactions between the holm oak *Quercus ilex* L. and its main acorn pests. Infested acorns were prematurely abscised before reaching their potential size. The volume of the acorns attacked by *Cydia fagiglandana* (Lepidoptera) was smaller than those attacked by *Curculio elephas* (Coleoptera); however, their weight did not differ because *Curculio* larvae consumed more cotyledon. For the same reason, embryo survival likelihood was not lower in *Cydia* acorns despite their smaller size. Delays of late summer rain reduced infestation by *Curculio*, as soil hardness hampers adult emergence from their underground cells. By contrast, late and scarce precipitations benefited *Cydia*; rainfall might hamper adult flight and eggs/L1 larvae survival. There was not a "zero-sum" effect, because the decrease of *Curculio* infestation rates was not fully compensated by an increase of *Cydia*. Under the longer droughts projected for the Mediterranean Basin, our results predict lower infestation rates and higher acorn survival likelihood. However, further studies including other environmental factors are needed to better forecast the net consequences for holm oak fitness.

Keywords: *Quercus ilex* L.; *Curculio elephas*; *Cydia fagiglandana*; climate change; longer droughts; Mediterranean Basin

1. Introduction

The forecasted worsening of pest impacts due to climate change is a main worry for forest ecologists and managers [1,2]. It may affect insect physiology, individual fitness and population growth, which in turn may provoke changes in insect community composition, ecological interactions and ultimately, ecosystem functioning [3–7]. In this context, we analyzed the potential consequences of the harsher droughts predicted for southern Europe [8,9] on holm oak *Quercus ilex* L. acorn pests.

Climate change effects on phytophagous insects can be direct or indirect. The first result from the consequences of environmental variables (e.g., rainfall, temperature) on insect fitness (e.g., insect development speed, reproduction rate, winter survival, etc.) [1,2,7,10–12]. Indirect effects are generally mediated by plants and include changes in food availability, plant community and/or structural composition, phenological mismatches between insects and their host plants, etc. [3,5,6,10,13]. Trophic specialists are especially vulnerable to indirect effects. This is the case with many phytophagous insects, which are strongly affected by any change in their host plants. These insects usually form assemblies of species

that feed on the same class of resources [14]. In these assemblies, some species are more susceptible than others regarding environmental changes [6,13].

In turn, changes in insect trophic guilds affect their host plants as well. In the case of antagonistic interactions, the negative effects on plant fitness differ between species [7]. In seed-feeding insects, for example, some species systematically predate more seeds than others in the same guild. A decrease in their populations translates into lower proportions of seeds destroyed when their place is not occupied by another species [6]. In other cases, population fluctuations are compensated, leading to a "zero-sum effect" in terms of seed predation: the species composition changes, but infestation rates remain unchanged [15].

The question of how climate change will affect oak *Quercus* spp. forests has attracted a lot of research attention in the last two decades. These trees are widespread over the Northern Hemisphere and support rich and diverse ecosystems. A large number of studies has analyzed the consequences of harsher droughts on oak growth, mortality or natural recruitment (see [16] for a review). However, we know very little about their potential effects on oak pests (but see [17]). In the case of acorn-borer insects, only one study [11] has addressed this subject and for just one insect species.

The communities of acorn-borer insects include species from different orders. The larvae of *Curculio* spp. (Coleoptera) weevils and *Cydia* spp. moths (Lepidoptera) complete their development within acorns feeding on the cotyledons. The identity at the species level may change, but the tandem *Curculio–Cydia* has been reported in oak forests worldwide (e.g., [15,18–22]). *Curculio* larvae are in most cases more prevalent than *Cydia* caterpillars; occurrence of other insect taxa is rare, with the exception of *Conotrachelus* spp. in North America [23].

Acorn-boring insects have received a lot of attention by pest researchers because they hamper natural regeneration [21,22,24]. Furthermore, in silvopastoral systems like the Iberian oak savannahs, these pests provoke economic losses, as acorns are a key food source for free range livestock [25]. Previous studies have shown that the lower summer rainfall predicted by climatic models may reduce weevil population sizes [11,12,26,27]. However, it remains unknown whether it will change the species composition of acorn-feeding guilds (i.e., relative prevalence of *Curculio* and *Cydia*).

In addition, if species composition changes, we still know little about the consequences for oak fitness. Such consequences would depend not only on infestation rates, but also on the fate of the infested acorns. *Curculio* larvae do not always kill the acorns; they can germinate if the embryo is not eaten, although the loss of cotyledon will reduce seedling size [21,28]. This might differ in the case of *Cydia*, depending on their method of feeding within the acorn. However, to the best of our knowledge, only two studies have compared embryo predation likelihood between acorn pests [15,19]. Moreover, it remains unknown whether the final acorn weight differs between those infested by *Curculio* or *Cydia*. This is key in oak silvopastoral systems like Iberian dehesas, as acorn biomass loss translates into lower productivity (i.e., meat production decreases) [29].

We carried out a multi-year monitoring of holm oak *Quercus ilex* acorn crops and pest insect community composition in three localities of central and western Spain. In addition, we carefully measured infestation rates and recorded the fate of each acorn. Relevant meteorological variables (i.e., late summer rainfall) were registered as well. Our general aim was to discover whether a reduction and delay of rainfall could change the relative prevalence of acorn pests and have further consequences on infestation rates. Our specific objectives were: (i) to test the differences between sound acorns and those infested by *Curculio* spp. and *Cydia* spp. in terms of: timing of acorn drop, acorn volume, final weight, cotyledon mass and embryo predation likelihood, (ii) to assess the effects of late summer rainfall (timing and total amount) on acorn infestation rates by *Curculio* and *Cydia*.

2. Materials and Methods

2.1. Study Areas

The data used in this study came from three localities of central and western Spain. The first one was Huecas, Toledo Province (39°59′ N, 4°13′ W), a flat agricultural land in which holm oaks are interspersed within cereal fields as isolated trees or in small plots (see [30] for a detailed description). In Huecas, acorn production and infestation rates by insects were monitored for six years (from 2008 to 2013) in 24 oaks. In three of those years, every single acorn was measured and opened to assess infestation, biomass loss and embryo predation. In addition, in two localities of the province of Cáceres: Guijo de Granadilla (40°8′ N, 6°7′ W) and Malpartida de Plasencia (39°56′ N, 5°58′ W), acorn production, infestation rates and the fate of infested acorns were measured for three years (2016 to 2018) in 16 and 8 trees, respectively. These last two places are human-made oak savannahs (so-called dehesas in Spain and montados in Portugal), traditional agroforestry systems in which free range livestock rearing is the main human use [25] (see Figure 1).
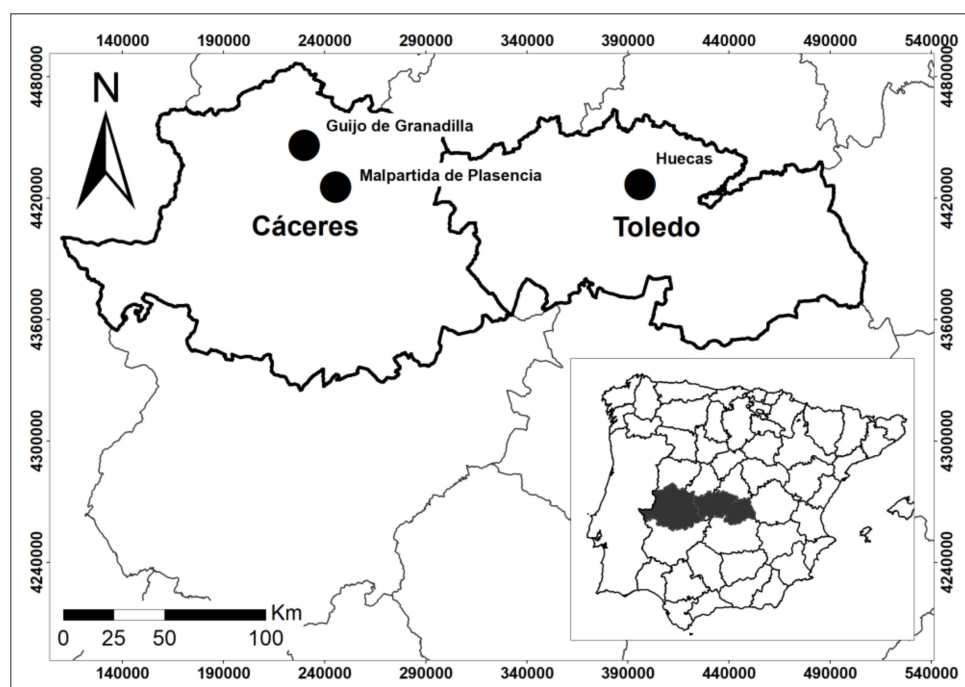

Figure 1. Location of the three study sites in the Iberian peninsula.

2.2. Study Species

The holm oak *Quercus ilex* L. is an evergreen oak distributed all over the western Mediterranean Basin, being the most widespread tree species in the Iberian Peninsula. Flowering occurs in spring and acorns grow throughout the summer and early autumn until mid-October. Sound acorns are dropped from then on until late December; those infested by insects are prematurely abscised and fall earlier on average [31]. Acorn size is extremely repeatable within the same holm oak, but very variable within and between localities. Acorn mass ranges from less than 1 to more than 10 gr [21]; their length ranges from scarcely 1 to more than 4 cm. Insect larvae feed on the cotyledons, which contain the reserves for seedling development [21,32]. Larval size at the end of their development also changes a lot among individuals, ranging from 30 mg to more than 120 [30]. Infested acorns can germinate and produce viable seedlings if the larvae do not predate the small embryo (length between 1 and 3 mm; R. Bonal unpublished data) located at the pointed end of the acorn [21,28,32] (Figure 2). The nutrient-rich acorns constitute an important food source in autumn and winter for free range livestock in traditional silvopastoral systems (i.e., dehesas) [25].
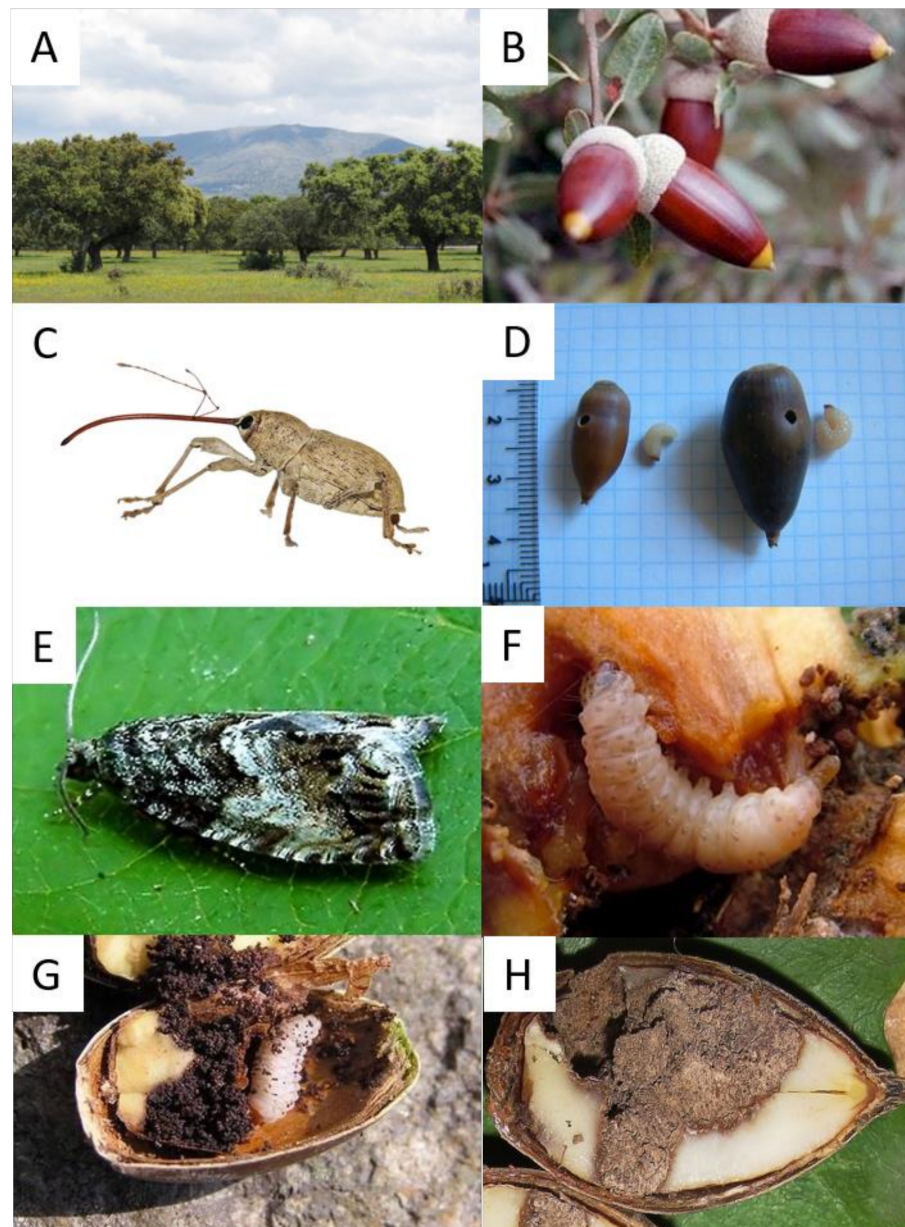
Figure 2. (**A**) One of the study areas with scattered holm oaks *Quercus ilex* L. (**B**) Acorns of *Quercus ilex* L. (**C,D**) *Curculio elephas* adult female and larvae. (**E,F**) *Cydia fagiglandana* adult and larva. (**I**) Acorn in which cotyledons have been mostly eaten and the embryo (located at the pointed end) has been predated. (**H**) Acorn with cotyledons only partially consumed in which the embryo has not been predated.

The main insects that feed on acorns are the specialist *Curculio* spp. weevils (Coleoptera: Curculionidae) and *Cydia* spp. moths (Lepidoptera: Tortricidae) [21,33]. In both cases, the larvae grow and complete their development within acorns feeding on the cotyledons, but other traits of their life histories differ. In the case of weevils, females drill the acorn shell with their long rostrum and introduce the oviscapt through the oviposition hole. Usually, a single egg is laid at each acorn, but larger clutches per seed may occur [34,35]. By contrast, adult moths do not drill the pericarp, but lay their eggs in the shoots close to the acorns. The newly hatched caterpillars get into the acorn by drilling the softer pericarp under the acorn cap (see Figure 2).

Curculio weevils are strictly univoltine. Larvae burrow underground just after finishing their development in October–November and remain in diapause within individual

earthen cells until the following year. Pupation takes place in underground refuges shortly before emerging as adults. The timing of adult emergence differs among species. In some it occurs in April–May, whereas in others it occurs from August onwards, once acorns are available in the canopies. Species with both types of strategies coexist in the same oak forests [36,37]. In our study areas we only recorded one species, namely *Curculio elephas*. *C. elephas* distribution is circum-Mediterranean, and it is very common in evergreen oak forests located in dry areas. Adults emerge from late August onwards favored by late summer storms that soften the soil surface [11,12,38] (Figure 2).

Among *Cydia* moths, *Cydia fagiglandana* is the most prevalent species in evergreen Mediterranean oak woodlands [33,39]. In our study area, we used DNA barcoding to identify a sample of 10 caterpillars and only recorded that species (unpublished data). *C. fagiglandana* is generally univoltine, but a small proportion of individuals may develop into adults and lay eggs in the same year [39]. *Cydia* caterpillars can walk, and their mobility is much higher than that of legless weevil larvae (Figure 2). They may leave the acorn within which they started their development and walk to get into another one through the exit hole drilled by a conspecific or weevil larvae [40]. Pupae overwinter slightly buried among the oak leaves on the ground [15] or in small crevices in the trunk (R. Bonal, personal observation). Adult flight starts in early summer (June–July) of the following year and oviposition takes place from August onwards, when grown acorns are available [39] (Figure 2).

2.3. Field Sampling and Acorn Classification

Seed traps were randomly placed under the canopies of all the study trees (hung from lower branches). The number of traps per tree was proportional to the canopy surface to cover a similar proportion in all trees (1 to 2%); canopy surface was calculated on an aerial picture using a geographical information system: ArcGIS [41]. This seed-trap-based methodology provided very reliable estimates of acorn crops (see [21]). Trap monitoring started in September and ended when the last acorns fell in late December. The content of the seed traps was collected every two weeks and taken to the laboratory, where acorns were classified as aborted or grown. In turn, all grown acorns were classified as sound or infested by insects (*Curculio* larvae or *Cydia* caterpillars).

Acorn classification was carefully carried out and confirmed following different procedures. In the first place, all grown acorns received a unique code and then were placed individually in plastic vials opened above and revised daily to record the number of larvae emerging from each one. Weevil larvae are legless and could not escape from the vials, which allowed an easy classification of the acorns attacked by *Curculio* (Figure 2). *Cydia* caterpillars were also frequently found in the vials, but sometimes they had left at the time of the daily monitoring. When we found an exit hole but no larvae, the acorn was tentatively classified as having been infested by *Cydia*; however, all acorns were subjected to further checking. *Cydia*-infested acorns could also be told apart by the shape of the exit hole drilled through the pericarp (irregular shape compared to the round ones chewed by *Curculio* larvae). Moreover, when the acorn was opened, we could inspect the size and shape of the excrements (dust-like in the case of *Curculio* larvae and small granules in *Cydia*) [42].

One month after the last larva had emerged, all acorns were oven-dried for 48 h at 80 °C. In order to calculate the volume, we measured acorn length and width with a digital caliper to the nearest 0.01 mm [36]. After that, to confirm a correct classification, we opened all of them to check whether there were dead larvae inside [43], excrements or any other sign of insect infestation. We also checked whether the embryo (located at the pointed end of the acorn) had been predated or not (Figure 2). Lastly, all acorns were weighted to the nearest 0.01 g. In the case of those infested by insects, we weighted only the acorn shell and the remains of cotyledons (if any) after carefully removing the excrements.

2.4. Acorn Crop and Infestation Rates

Infestation rates by *Curculio* and *Cydia* were computed for each tree as the ratio between the number of acorns of each class divided by the total number of grown acorns (sound plus infested by both types of insects). We also calculated the total number of grown acorns produced by each tree by multiplying the number of acorns produced per square meter by the surface of the canopy.

2.5. Meteorological Data

We used the daily precipitation records from the closest meteorological station for each of the three study sites. For Huecas, province of Toledo, the data came from the meteorological station of Toledo ($39°53'$ N, $04°02'$ W). For the two study sites at the province of Cáceres, Guijo de Granadilla and Malpartida de Plasencia, the data came from the stations of Guijo de Granadilla ($40°11.5'$ N, $06°09.9'$ W) and Plasencia ($39°58.7'$ N, $06°09.0'$ W), respectively. The distances between the meteorological stations and each of the three study sites were thus 19.18 km, 7.67 km and 16.41 km, respectively. Although the distances differed, they are not likely to have affected the results. There are no mountains or other significant barriers between the study sites and the meteorological stations that might change rainfall patterns at that scale.

We considered the same variables that were considered in previous studies on the effects of meteorology on *C. elephas* phenology [11,12,27], namely total rainfall during the emergence period and the date of the first late summer rain. Total rainfall corresponds to the summed rainfall (L/m^2) during the period in which most adult *Curculio* emergence is concentrated, from late August to October. This is also the period in which *Cydia* females are ovipositing; hence, we considered it appropriate to use the same variable for both. The first late summer rain is the date from August 4th onwards in which at least a daily precipitation of 1 L/m^2 was recorded (please see [11] for a detailed explanation of the two variables).

2.6. Statistical Analyses

We used linear mixed models (LMMs) to test the differences between sound acorns and those infested by *Curculio* spp. and *Cydia* spp. in terms of: timing of acorn drop, volume and final weight. The fixed independent factor had three levels (sound, *Cydia*, *Curculio*). We also tested the differences in cotyledon mass with another LMM including the same fixed factor but adding a continuous covariate (acorn volume in cm^3). By doing this, we could assess how much biomass infested acorns had lost with respect to sound ones of the same volume. In all LMMs, the tree and site identities were the random factors (the former nested within the latter). The likelihood of embryo predation was tested only between acorns infested by *Cydia* and *Curculio*. To do so, we performed a generalized linear mixed model (GLMM) with binomial family (dependent variable: embryo predated/not predated). The fixed independent factor had two levels (*Cydia*–*Curculio*). Tree and site identities were the random factors (the former nested within the latter).

To assess the effects of late summer rainfall (timing and total amount) on acorn infestation rates, we performed three generalized linear mixed models (GLMMs) (binomial family). In all of them, tree and site identities were the random effects (the former nested within the latter). The dependent variables in each of the three GLMMs were the proportion of grown acorns infested by: (i) *Cydia*, (ii) *Curculio* and (iii) pest insects as a whole (*Curculio* plus *Cydia*). The independent variables in all GLMMs were the total rainfall during insect emergence period, the date of the first late summer rain and the total number of acorns produced by the tree (log. transformed). In addition, to test for any interference between pest insects, we included *Cydia* infestation rates as a predictor variable of *Curculio* infestation rates and vice versa.

All models were validated testing homogeneity and normality of the residuals. Furthermore, all post hoc tests were made with Tukey test. Additionally, all data analyses

were performed with the statistical analysis program R Core Team [44] and the following libraries were used: plyr [45], ggplot2 [46], nlme [47], lme4 [48] and multcomp [49].

3. Results

Using a sample of more than 6000 acorns, we assessed that acorns attacked by insects were prematurely abscised and fell, on average, more than one month earlier than sound ones (mean dates: 26 November for sound acorns; 21 October and 20th for those infested by *Curculio* and *Cydia*, respectively) ($F_{2,6285}$ = 1135.939; p < 0.001). Post hoc tests to assess pairwise differences in mean dates of abscission between acorns of the three categories were significant when sound acorns were compared with those infested by *Cydia* (estimate$_{Cydia}$ = 30.077, Z_{Cydia} = 35.245, P_{Cydia} < 0.001) or *Curculio* (estimate$_{Curculio}$ = 28.940, $Z_{Curculio}$ = 41.813, $P_{Curculio}$ < 0.001). By contrast, the *Curculio–Cydia* comparison was not significant (p = 0.418).

Acorn volume (cm^3) varied significantly between the three categories of acorns ($F_{2,6285}$ = 254.719; p < 0.001). Sound acorns were the largest, followed by those infested by *Curculio*; acorns attacked by *Cydia* were the smallest (Figure 3). All post hoc pairwise comparisons were significant (see Table 1).
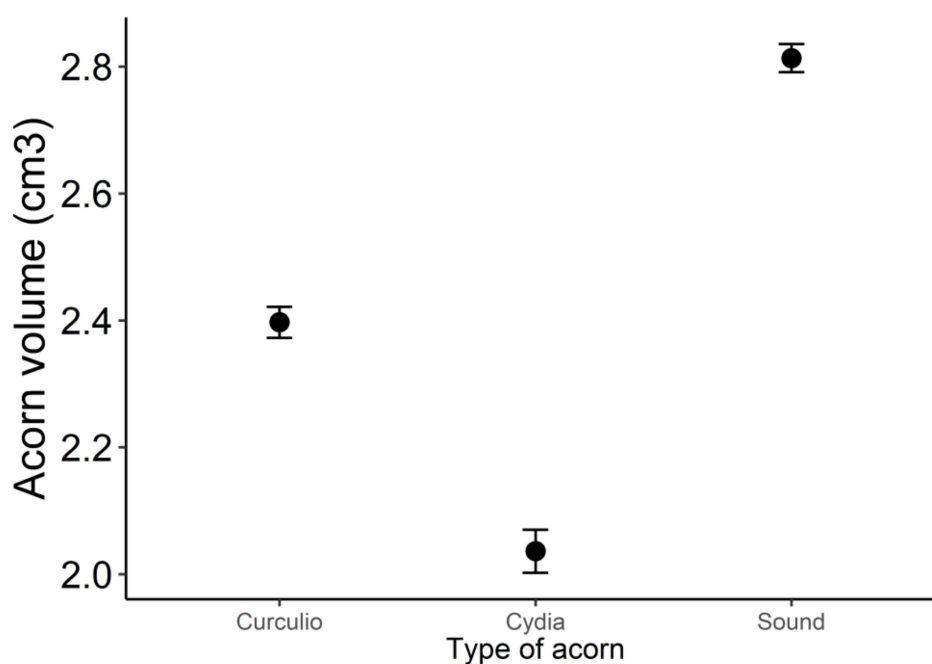

Figure 3. Acorn volume (cm^3) of the three different types of grown acorns. Dots show the mean per category and error bars the standard deviation of the mean.

Table 1. Results of the Tukey post hoc pairwise comparison test in acorn volume analysis.

Type of Acorn	Estimate	Z	p
Cydia–Curculio	−0.318	−8.266	<0.001
Sound–Curculio	0.440	14.932	<0.001
Sound–Cydia	0.758	20.899	<0.001

Acorn total dry weight (in grams, shell plus cotyledon) also differed significantly among categories ($F_{2,6276}$ = 1462.573; p < 0.001). Sound acorns were twice as heavy as those infested by acorn-borer insects (mean ± SE: 2.43 ± 0.02 g vs. 1.16 ± 0.02 g -*Curculio*- and 1.17 ± 0.03 g -*Cydia*-). All pairwise post hoc comparisons between sound acorns and the rest were significant (estimate$_{Curculio}$ = 1.194; $Z_{Curculio}$ = 48.896; $P_{Curculio}$ < 0.001; estimate$_{Cydia}$ = 1.139; Z_{Cydia} = 37.894; P_{Cydia} < 0.001). On the contrary, there were no

significant differences between acorns infested by *Curculio* and *Cydia* (estimate = 0.055; Z = 1.727; *p* = 0.193).

In spite of their larger volume, *Curculio*-infested acorns were not heavier than those attacked by *Cydia* because weevil larvae consumed more cotyledon. Cotyledon mass increased with acorn volume ($F_{1,4490}$ = 6323.767; *p* < 0.001), but it differed between acorn types ($F_{2,4490}$ = 2959.132; *p* < 0.001). For any given acorn volume, cotyledon mass was higher in sound acorns, in which nothing had been consumed by insects. Moreover, there were differences between those infested by insects as well. For a given volume, the weight of the cotyledon remains once the larvae had finished feeding was larger in acorns infested by *Cydia* (Figure 4). The interaction between the fixed factor and the covariate was significant too ($F_{2,4490}$ = 340.612; *p* < 0.001; Figure 4).
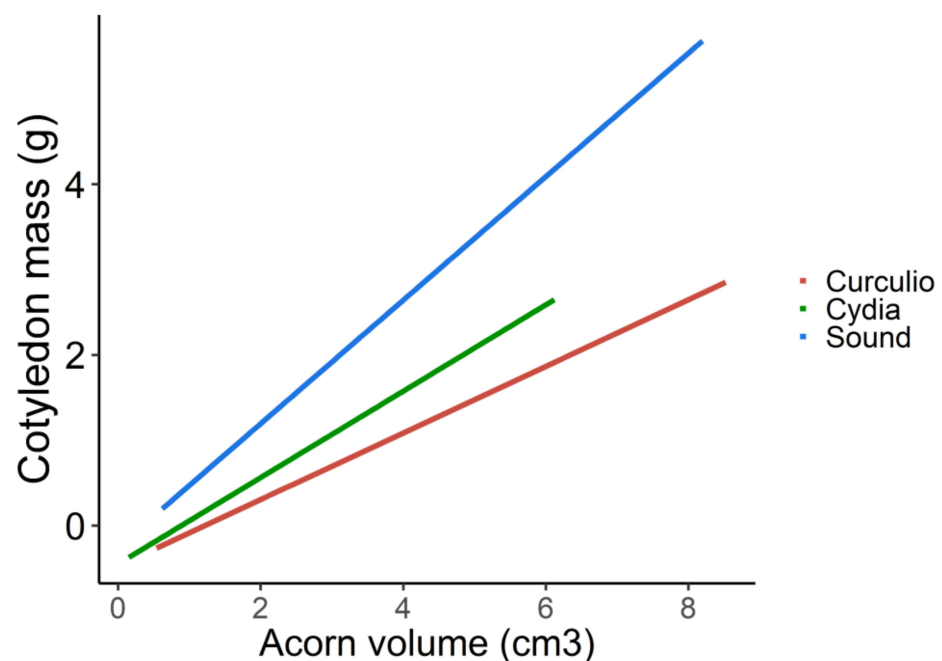

Figure 4. Relationship between acorn volume (cm^3) and cotyledon dry mass (g) in the different types of acorns. Blue, green and red lines correspond to sound acorns and those infested by *Cydia* and *Curculio*, respectively.

In 68.24% of *Curculio*-infested acorns, the embryo was killed (N = 1851); this percentage was lower in those attacked by Cydia: 54.89% (N = 891). Embryo survival likelihood differed between types of acorns (Chi^2 = 81.933; *p* < 0.001) and was lower in acorns with a smaller volume (estimate = 0.0007; Z = 10.483; *p* < 0.001). The interaction between the fixed factor and the covariate was significant (Chi^2 = 18.621; *p* < 0.001). In the acorns infested by *Cydia*, acorn survival likelihood increased with acorn volume more quickly (Figure 5).

Pooling all years and sites together, acorn infestation rates by *Curculio* were twice those by *Cydia* (Mean ± SE: 31.15 ± 2.83 and 15.60 ± 1.78, respectively). Infestation rates by *Curculio* were lower when late summer storms were delayed. Infestation rates by *Curculio* depended on the timing of the first rains (Chi^2 = 169.65; *p* < 0.001) and acorn production (Chi^2 = 216.07; *p* < 0.001). It was negatively related to acorn crop size (estimate = −0.470; Z = −14.700; *p* < 0.001) and the date of the first late summer rains (estimate = −0.033; Z = −13.030; *p* < 0.001; Figure 6). *Curculio* infestation rates were unaffected by the total amount of rainfall during the emergence period and the proportion of acorns attacked by *Cydia* (all *p* > 0.05). By contrast, infestation rates by *Cydia* increased when late summers were dry. Infestation rates by *Cydia* depended on the first rain (Chi^2 = 27.232; *p* < 0.001), the amount of rain (Chi^2 = 8.160; *p* = 0.004) and acorn production (Chi^2 = 93.625; *p* < 0.001). *Cydia* infestation rates were negatively related to acorn crop size (estimate = −0.358;

Z = −9.676; *p* < 0.001). However, contrary to *Curculio*, they were positively related to the date of first late summer rainfall (estimate = 0.020; Z = 5.218; *p* < 0.001; Figure 6) and negatively related to the total amount of rain during *Cydia* activity period (estimate = −0.003; Z = −2.857; *p* = 0.004). Lastly, *Curculio* infestation rates had no effect on *Cydia* infestation rates (*p* = 0.355).
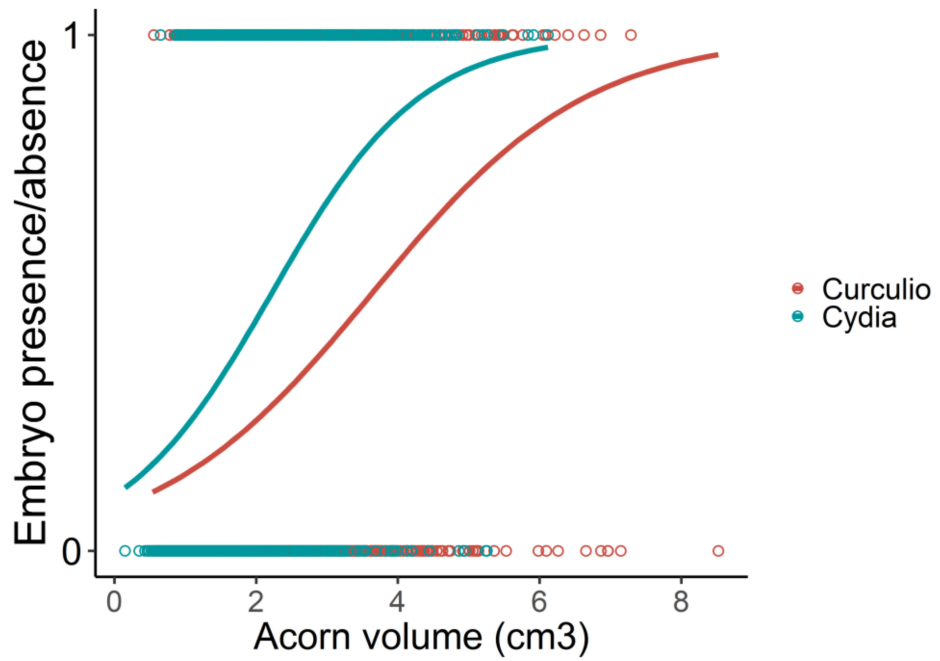

Figure 5. Relationship between acorn volume and embryo survival in acorns infested by *Curculio* and *Cydia*. The *x*-axis represents the acorn volume in cm^3. The *y*-axis depicts embryo survival: (0–embryo killed. 1–embryo not killed). Red dots correspond to *Curculio* acorns and blue ones to those infested by *Cydia*.
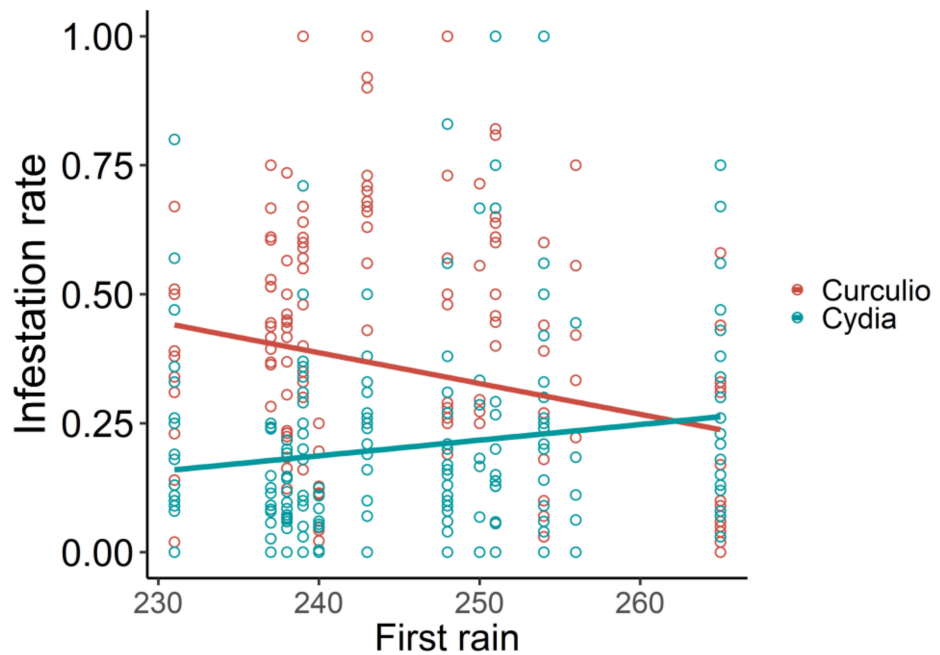

Figure 6. Relationship between the date of the first late summer rains and acorn infestation rates by *Curculio* and *Cydia*. First late summer rain is the date from 4 August onwards in which at least a daily precipitation of 1 L/m^2 was recorded. Julian dates (day 1 = 1 January) are used.

When we pooled together all insect-infested acorns (*Curculio* plus *Cydia*), infestation rates greatly depended on acorn production (Chi^2 = 395.014; *p* < 0.001) and first rain (Chi^2 = 31.466; *p* < 0.001). Rates were negatively related to crop size (estimate = −0.711131; Z = −21.743; *p* < 0.001) and the date of the first late summer storms (estimate = −0.020890; Z = −9.022; *p* < 0.001). The total amount of rain during the insect activity period had a marginally significant effect (estimate = −0.002; Z = −1.920; *p* = 0.055).

4. Discussion

Acorns attacked by *Cydia* had a smaller volume (cm^3) than those infested by *Curculio*, despite their overlapping drop dates. However, their weight once the larvae left did not differ because *Curculio* larvae consumed more cotyledon mass than *Cydia* caterpillars. Accordingly, embryo survival likelihood was lower in acorns infested by *Curculio*. Meteorology had an effect on insect infestation rates. The total amount of rainfall significantly reduced acorn attack by *Cydia*, but had no effect on acorn attack by *Curculio*. By contrast, the delay of late summer rainfall reduced infestation rates by *Curculio*, whereas it increased acorn infestation by *Cydia*. However, *Cydia* increase did not compensate *Curculio* decrease, and total infestation rates (pooling together all insect-infested acorns) decreased in those years in which late summer storms started later.

The volume of the acorns infested by *Curculio* or *Cydia* was smaller than that of sound ones (dropped one month later). Strikingly, acorn volume also differed between acorns attacked by *Curculio* and *Cydia*, although they were dropped in the same period. Numerous studies have reported the premature abscission of infested acorns compared to sound ones [20,43,50,51]. Insects oviposit into acorns, thus stopping their growth before completing their development, what explains their smaller volume [31,52]. Thus, the smaller volume of the acorns infested by *Cydia* could suggest an earlier reproductive phenology compared to *Curculio*. However, their largely similar abscission dates do not match with this hypothesis, unless the period from infestation to abscission is longer in the case of *Cydia*. The mechanisms behind this differential response, if they exist, remain unknown.

The differences in volume (cm^3) between infested acorns might also be a consequence of differences in acorn selection. Reports from both chestnut tree orchards (*Cydia splendana* and *Curculio elephas*) [53] and holm oak forests (*Cydia fagiglandana* and *Curculio elephas*) [39] have shown an earlier emergence of adult moths but overlapping acorn use by the larvae of the two types of insects. *Cydia* moths oviposit earlier, but it is the first instar caterpillar that enters into the acorn, and egg hatching takes approximately 10 days. In the case of *Curculio elephas*, females oviposit into the acorns as soon as they emerge and eggs hatch in five days [53]. *Cydia* caterpillars depend on acorn cotyledons for completing their development; however, they may move between acorns during the growing period [40]. By contrast, the legless weevil larvae have to complete their development within a single acorn. *Curculio* females should thus be more selective and choose bigger acorns (i.e., larger volume), as seed size could constrain the growth of their offspring and future fitness [34,35]. Nonetheless, further acorn selection tests are necessary to confirm this hypothesis.

In spite of their smaller volume, the mass of the acorns infested by *Cydia* was not lower compared to those attacked by *Curculio*. Weevil larvae consumed larger amounts of cotyledons and the differences disappeared. Multi-infestation is frequent in *Curculio* weevils, especially when seed availability is lower [19,31,34]. When more than one weevil larvae share the same acorn, cotyledon depletion is not rare and larval weight can be constrained by acorn size [19,35]. By contrast, *Cydia* caterpillars develop singly, multi-infestation being extremely rare [19,33,53]. Conspecific aggressions exclude other caterpillars (see [33] and references therein), and *Curculio* females avoid *Cydia*-infested acorns for oviposition [53]. Single *Cydia* caterpillars rarely deplete the cotyledons, and their weight is not constrained by acorn size [19].

Embryo predation risk was higher the smaller the acorn volume but, overall, it was greater in acorns infested by *Curculio*. *Curculio* weevils start boring the acorn from the

bottom and, in large acorns, they are less likely to predate the embryo on the opposite side [19,21]. *Cydia* caterpillars start feeding from the same side, but they are satiated earlier at the seed level [21]. In acorns with a smaller volume, the embryo is more likely to survive if it is infested by *Cydia* than if it is attacked by *Curculio*. Even in forests in which acorns have a smaller volume than in our study site, total embryo survival likelihood is significantly higher in those infested by *Cydia* [15].

The delay of late summer rainfall decreased infestation as it hampers adult emergence. Adult *Curculio elephas* need the rain to soften the soil and facilitate emergence from their underground earthen cells [11,38]. A previous study, based on a single site and a shorter time series, showed that infestation rates are positively related to the total rainfall throughout the emergence period [27]. The present results show that it is the rainfall timing that has a stronger effect. Moreover, after including it in the model, the effect of total rainfall on infestation rates was not significant. Total rainfall is, however, positively correlated with the total number of *C. elephas* adults that emerge [11,12]. Hence, a plentiful late rain may trigger an abundant adult emergence, but not necessarily massive oviposition. The onset of adult emergence may change a lot between years (up to one month) [27] and, later in the season, the suitability of acorns for oviposition might decrease (e.g., increased hardness; see [54] for the case of *Curculio nucum* and the hazelnuts of *Corylus avellana*). Further analyses could test whether the relationship between the number of *C. elephas* adults and acorn infestation rates is affected by the timing of late summer rain.

Contrary to *Curculio*, infestation rates by *Cydia* increased when late summer rainfall was lower and started later; however, the underlying mechanisms differ. *Cydia* pupae are not buried underground, but are among the litter beneath oak canopies [15]. They do not need the rain to soften the soil. On the contrary, storms could have negative effects on adults, eggs and newly hatched caterpillars. Rain hinders small insect flight [55] and reduces adult activity. In the case of eggs and first instar caterpillars, storms may provoke high mortality rates (see [56] and references therein). *Cydia* oviposition takes place mainly in September, and there is a period of two weeks in which eggs and L1 larvae are unprotected (on the oak shoots). This might explain the higher acorn infestation rates by *Cydia* in dry years.

Acorn infestation rates by borer insects as a whole (*Curculio* plus *Cydia*) decreased significantly, but slightly, when late summer storms were delayed. The increase of infestation rates by *Cydia* did not compensate for the decrease of those by *Curculio*; hence, the effect was not "zero-sum" (sensu [15]). According to data on other seed feeding insects [6], the population of one species did not "fill the gap" left by the other. Under strong interspecific competition such effects could have appeared; however, this does not seem to be the case with acorn-borer insects. Reports on the same species in chestnut trees *Castanea sativa* have not found interspecific interference at the tree level [53]. In oaks, although infestation rates may be high, they rarely get to 100% [12,21]. The present results contrast with a previous study, in which the low *Curculio* numbers in fragmented oak forests were compensated by the increase of *Cydia* moths [15]. The lower total infestation rates in those oak plots (20%) probably facilitated the species substitution.

In our study sites, as in most oak woodlands worldwide, *Curculio* weevils were the main acorn-borer insects [18–20,33]. However, our results show that such dominance may change if late summer rainfalls arrive late. In the Mediterranean region, current projections forecast longer and drier summers [8,9]. Under this scenario, more acorns might escape predation; moreover, the substitution of *Curculio* by *Cydia* would not reduce embryo survival likelihood or the mass of infested acorns. Oak recruitment might be favored and, in the case of Iberian oak savannahs, meat production might increase along with a decrease in acorn infestation [29]. However, there are a number of factors to explore before making such a statement.

In the first place, it remains unknown whether the effect of rainfall can be extrapolated to other climates. Acorn predation by insects occurs in oak forests worldwide, including tropical and subtropical areas with very different climatic conditions [22,57]. In addition,

although rain delay can reduce insect infestation, harsh summer drought increases acorn abortion [58] and hampers seedling recruitment [50]. The net effects of environmental changes on ecological interactions are difficult to predict due to the number of co-occurring factors. However, according to other studies on acorn production in temperate forests [59], our results show that not all the consequences might be detrimental for oak fitness. Further research on the outcome of climate change should contemplate this double perspective.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are not publicly available due to their usage in the ongoing study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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